# One-dimensional Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

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## Introduction

### Technical and Developmental Details of Basic Technique

Any ion will undergo electrophoresis to migrate in an electric field. Proteins are complex polyions with a net charge at all pH values other than its isoelectric point. Problems associated with convective disturbance in free solution led early researchers to consider various supporting media for electrophoresis such as paper, cellulose acetate and various thin layer materials where the separation depends largely on the charge density at a given pH. The reader should refer to the separate articles on Electrophoresis Theory and Cellulose Acetate Electrophoresis for further details. Other early workers considered the properties of various gels where the pore size approximates the size of the protein molecules themselves leading to a separation based on both charge and molecular size. The extent of molecular sieving depends on the pore size of the gel being used. For example, the pore size of agarose gels is sufficiently large that sieving of most proteins is minimal, whereas larger DNA molecules are sieved very well. Again, for a discussion of this refer to the separate article on Agarose Electrophoresis.

The pore size of polyacrylamide gels may be changed in a systematic and reproducible fashion by varying the percentage of monomer and crosslinker to give a matrix which maximizes the molecular sieving effect for a wide range of proteins and the reader should refer to the separate article on Polyacrylamide Gel Electrophoresis (PAGE) for a discussion on varying porosity in this medium.

Native proteins, however, often occur as multiple supramolecular assemblies of many peptide subunits in different configurations affected by noncovalent bonding, particularly in the case of membrane proteins. Shapiro *et al.* (1967) first demonstrated the potential of the superior protein dissociating qualities of sodium dodecyl sulfate (SDS) in an electrophoretic system designed to separate individual polypeptides on the basis of their molecular weight alone but the definitive publication in this area is undoubtedly that of Laemmli (1970) who first combined a discontinuous buffer system (see separate article on Discontinuous Electrophoresis) with the use of SDS in sample preparation and gel electrophoresis. Protein complexes are solubilized and dissociated with such high efficiency in 2% SDS and 5% mercaptoethanol that typically over 90% of the protein in a crude lysate will enter the gel matrix and be resolved.

In the discontinuous system, proteins are dissolved by denaturing treatment at 100°C with the dissociating agents SDS and mercaptoethanol in a Tris-HCl buffer at pH 6.8. Gels are constructed in two stages both containing 0.1% SDS. The separating gel in the original Laemmli publication was formed using 30% stock acrylamide monomer with 0.8% bisacrylamide as a cross-linker. A final solution was made to 8 or 10% acrylamide containing 0.375 M Tris-HCl pH 8.8. The resolving (separating) gel is polymerized using tetramethylenediamine (TEMED) (catalyst) and ammonium persulfate (free radical initiator). A 'stacking' gel (at 3% acrylamide) is then cast on top of the resolving gel in the same manner but containing 0.125 M Tris-HCl at the same pH as the buffer in which the protein mixtures were dissociated (pH 6.8). The electrode buffer contains 0.025 M Tris/0.192 M glycine to a pH of 8.3 also with 0.1% SDS. Upon electrophoresis, protein anions in the form of rod-shaped SDS complexes are compressed in the stacking gel between the leading chloride ions and the trailing glycinate ions which, because of the pH difference between buffer systems, progressively close the gap as electrophoresis proceeds. (Again, see the separate articles on Discontinuous Electrophoresis and Isotachophoresis). The result is a concentration or stacking of the SDS–protein anions as extremely sharp bands ( $\approx$ 5–10 μm) behind the leading chloride ion in strict order of mobility. These complexes then enter the separating gel and since, supposedly, the charge–mass ratio is invariant (see later for a caveat) are separated by molecular sieving according to their molecular size only. Gels of particular acrylamide concentration and therefore pore size may be calibrated using standard proteins of known molecular weight. By extrapolation, reliable molecular weight estimates of large numbers of polypeptides in a complex mixture may be obtained. Proteins are fixed in the gel after electrophoresis using a 50% trichloroacetic acid solution and stained in a Coomassie blue solution. Radiolabelled proteins were also detected by autoradiography. For a more detailed

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discussion please refer to the separate article on Detection Techniques, Staining, Autoradiography and Blotting.

In the original Laemmli procedure, gels were electrophoresed in small glass cylinders and since the absolute mobility of SDS-polypeptides varied slightly from gel to gel the relative mobility of standard and unknown bands was calculated as the ratio between the mobility of the protein and the mobility of the tracking dye, bromphenol blue (BPB), which travels with the SDS-micelle front behind the leading buffer front.

A major improvement on the original procedure uses rectangular slab gels of uniform thickness (typically 0.5–1.5 mm) with sample wells (typically 10–30) set into the stacking gel. Electrophoretic apparatus was originally constructed according to a variety of *ad hoc* patterns, in house, from perspex, using gels polymerized between notched glass plates, although now most laboratories use commercially available equipment. The use of such equipment (see the separate article on Slab Gel Electrophoresis: Equipment) has led to highly standardized reliable separations since standards and unknowns may be run under identical conditions. The apparent molecular weights of unknowns may be obtained by extrapolation from a plot of log MWt vs. mobility of standard proteins. **Figure 1** shows how individual

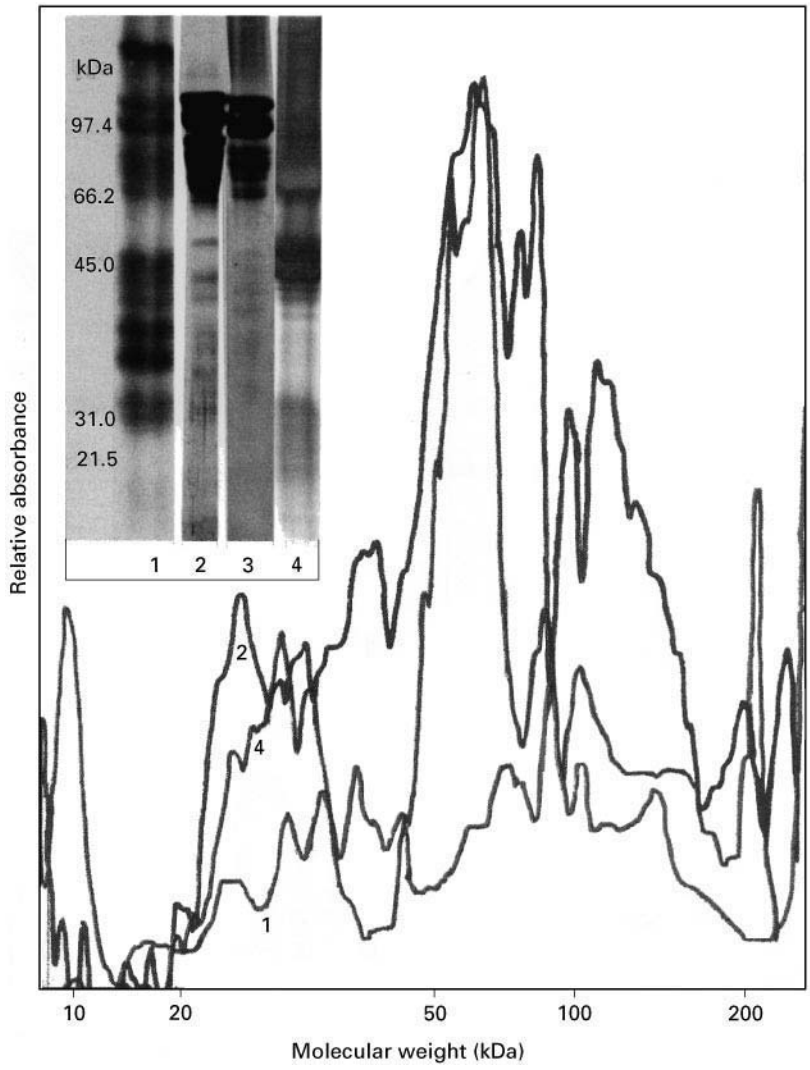


**Figure 1** (See Colour Plate 41). The inset shows the separation of human head hair proteins using 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis with the point of migration of standard proteins to the left under the heading kDa: lanes 1–3 *S*-carboxymethylated proteins; lane 4 reduced non-*S*-carboxmethylated proteins: lane 1 $^{14}$C Autoradiograph; lanes 2 and 4, silver stain; lane 3, Coomassie stain. The main body of Figure 1 shows a densitometric profile of the separated lane 1 (autoradiograph $^{14}$C-*S*-carboxymethylated, green). Lane 2 (silver stain, *S*-carboxymethylated, red); lane 4 (silver stain, non-*S*-carboxymethylated). Molecular weights were extrapolated from a plot of log MWt vs. relative migration of standard proteins.

zones in a complex mixture of human hair keratins may all be assigned an apparent molecular weight and how the entire molecular weight profile is apparently shifted 20–40 kDa higher after *S*-carboxymethylation.

Although several alternative sample extraction and buffer systems are available (see later), the use of the basic Laemmli system has proved so robust and reliable that it has revolutionized protein characterization in complex mixtures to the extent that proteins of widely differing function are routinely described according to their apparent molecular mass on Laemmli SDS-PAGE. The explosive increase in the use of this technique across a comprehensive range of the biological sciences is illustrated in **Figure 2** showing the number of citations each year of the Laemmli (1970) publication.

I will now discuss subsequent major methodological developments of the basic Laemmli protocol.

## Other SDS-PAGE Systems

The SDS-PAGE technique may also be performed with the simpler continuous phosphate buffer system of Weber and Osbourne, although dilute samples are not concentrated by stacking as they are in the discontinuous system of Laemmli. In general, however, very dilute protein samples may be concentrated by precipitation with trichloroacetic acid or acetone prior to loading.

Separations of peptides and protein mixtures ranging in molecular weight from 300 kDa down to about 10–12 kDa may be optimally achieved using resolving gels of different fixed acrylamide concentrations as, for instance, shown in **Figure 3**A (7.5%) or B (10%) for the separation of phosphoproteins during the invasion of human red blood cells by the malarial parasite. Alternatively, a gradient mixer may be used to produce resolving gels with a linear or concave concentration gradient.

Peptides of less than 10 kDa are not resolved using the normal Laemmli system even at maximal acrylamide concentration so a variety of modified buffer systems have been introduced to allow the separation of, for example, cyanogen bromide and proteolytically cleaved peptides for structural analysis of proteins. The original Swank and Monkres system uses SDS/urea and is capable of resolution down to 2 kDa, although some undesirable modifications of amino acids may take place in the presence of urea. Other buffer systems designed to fractionate low molecular weight peptides such as the tricine and the modified Laemmli system have been more recently introduced to address this problem. For a detailed technical discussion of the preparation of gels using these various buffer systems one should refer to one or more of the following generally excellent technical guides available (*Current Protocols in Molecular Biology*; *Gel Electrophoresis of Proteins – A Practical Approach*; *Protein Purification, Principles – High Resolution Methods and Techniques*).

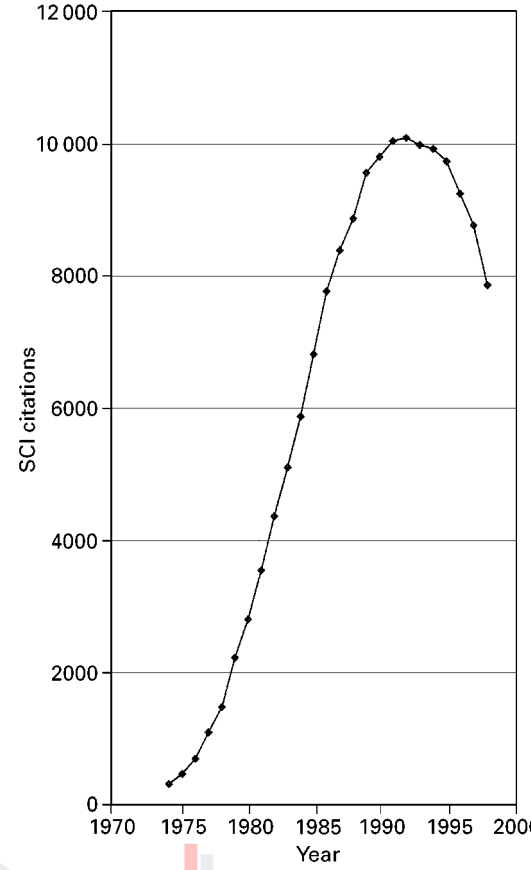


**Figure 2** The number of SCI citation of the original Laemmli, U.K. (1970) paper on sodium dodecyl sulfate–polyacrylamide gel electrophoresis for each year from 1974 (when SCI started).

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## Identification of Resolved Protein Zones

Protein bands may be directly visualized by scanning unstained unfixed gels at $A_{280}$ but most workers employ a protein stain after fixation. Coomassie R-250 dye binds noncovalently to proteins giving deep blue bands on a clear background after diffusion (see **Figure 5**) or electrophoretic destaining. Alternatively, fixed proteins may be revealed by the precipitation of silver granules from an alkaline silver nitrate solution. The procedure is more complicated than the dye-binding protocol but can be up to 100 times more

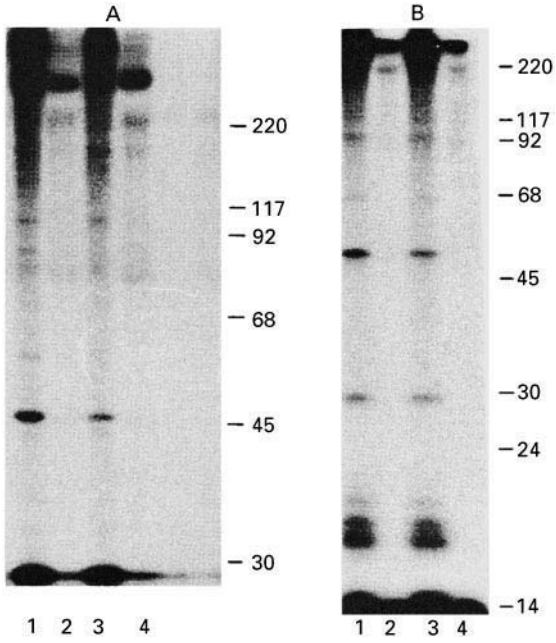


**Figure 3** Sodium dodecyl sulfate–polyacrylamide gel electrophoresis showing protein phosphorylation in human blood cells parasitized with the malarial organism *Plasmodium falciparum* using continuous metabolic labelling with $^{32}$P phosphate. A, 7.5% Resolving gel; B, 10% Resolving gel. Mobility of molecular weight standards indicated in kiloDaltons to the right of gel A and gel B. Lane 1, parasite pellet (mature shizonts) 36 h post label; lane 2, infected red cell ghost 36 h post label; lane 3, parasite pellet (new rings) 50 h post label. Lane 4, infected red cell ghost 50 h post label.

sensitive. Several commercial kits are now available, ensuring the robust reproducibility of the once notoriously fickle silver staining technique. Because of this and the added sensitivity the silver staining technique has now become standard in most laboratories. Proteins vary markedly in their affinity for either Coomassie dye or silver. The insert to **Figure 1** (lanes 2 and 3) shows a comparison of the proteins separated from human hair after solubilization and carboxymethylation using either silver stain or Coomassie stain respectively. The differing sensitivities are obvious. Lane 1 shows the same proteins labelled with $^{14}$C iodoacetic acid and revealed by autoradiography. Note that the lower molecular weight zones are very strongly labelled whereas they stain relatively weakly with silver (lane 2) and very poorly with Coomassie dye (lane 3).

## Detection of Radiolabelled Proteins

Proteins may be radiolabelled during synthesis in the presence of labelled amino acids. $^{35}$S methionine is commonly used in this regard. **Figure 4**, for instance, shows biosynthetic labelling in human lymphocytes before and after heat shock. $^{3}$H and $^{14}$C labelled amino acids are also used as is $^{32}$P phosphate (see Figure 3) to label phosphoamino acids. $^{35}$S, $^{14}$C and $^{32}$P may be detected by direct autoradiography by placing fixed dried gel slabs against suitably sensitized X-ray film. The sensitivity of detection depends, on the level of incorporation of the particular

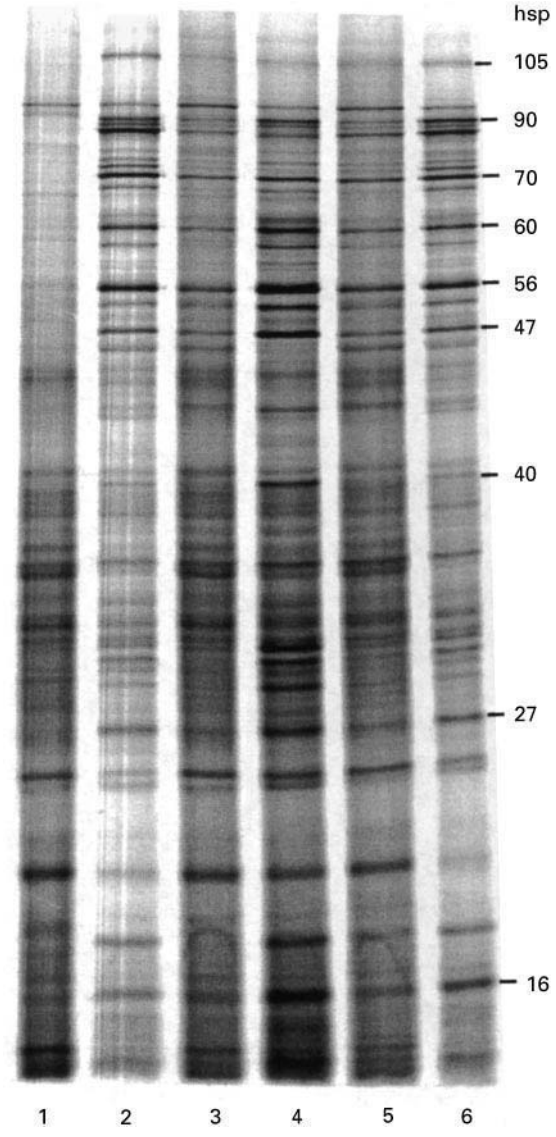


**Figure 4** A 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis separation of $^{35}$S methionine-labelled proteins from human lymphocytes after different recovery periods from 1 h heat shock at 42°C. Control cells from the same individual were maintained at 37°C. Lane 1, 2 h control; lane 2, 2 h after heat shock; lane 3, 3-h control; lane 4, 3 h after heat shock; lane 5, 4-h control; lane 6, 4 h after heat shock. Numbers to the right of the gel indicate the position of major heat shock proteins (hsp) induced after heat shock. Hsps are routinely classified according to their apparent molecular weight (105, 90, 70 kDa, etc.) after one-dimensional SDS-PAGE. (Thanks to my PhD student D. Visala Rao for this gel.)

amino acid into the particular protein zone but also on the isotope itself. For example after a 24-h exposure about 300–500 d.p.m.cm$^2$ of $^{32}$P will give a visible band whereas about tenfold this level of radioactivity is required to produce a visible band using $^{35}$S or $^{14}$C label. $^3$H label is not detected because the low $\beta$-emissions do not penetrate the gel matrix. A fluor may be introduced into the gel matrix prior to gel drying to detect $^3$H-labelled proteins as well as to improve the sensitivity to $^{14}$C and $^{35}$S.

Proteins may also be labelled post synthetically using such reagents as $^{14}$C iodoacetic acid which preferentially labels available sulfhydryl groups (see **Figure 1**, insert lane 1) and $^{125}$I which preferentially labels available tyrosine groups. Many laboratories use phosphoimaging whereby the radioactive proteins in the gel excite a phosphorescent screen and the number of excitation events is directly digitized. Although this instrumentation is rather expensive it obviates the need for X-ray film and gives results in hours rather than days. Effective concentrations are said to be linear over six orders of magnitude whereas the linear range for X-ray film detection rarely covers one order of magnitude. For further details see the separate article on Detection Techniques: Staining, Autoradiography and Blotting. Radioactivity may also be quantified in gels after slicing and solubilization. In this case, a generally useful labile crosslinker *N*,*N*$^1$-diallyltartardiamide (DATD) is often used in place of bisacrylamide.

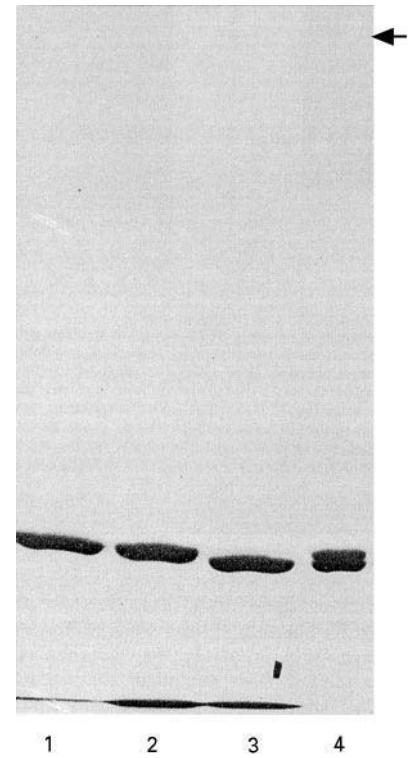


**Figure 5** Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of purified human carbonic anhydrase I. The horizontal arrow indicates the interface between the 4% stacking gel and the 10% resolving gel. (1) Purified Caucasian enzyme; (2) purified normal component from isoelectric focusing; (3) purified variant component from isoelectric focusing; (4) purified heterozygote mixture before resolution of normal and variant components. Reproduced with permission from Jones GL and Shaw DC (1982) *Biochemical Genetics* 20: 943.

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## Identity of Individual Protein Zones

The appearance of single discrete zones on SDS-PAGE, sometimes used as an indicator of homogeneity in protein purification (see **Figure 5**), does not preclude the possibility of multiple comigrating protein species with differing independent functions. Zones may be identified immunologically after Western blotting onto a suitable matrix and again the reader should refer to the separate article on Detection Techniques: Staining, Autoradiography and Blotting in this series for further details.

Although the process of the SDS-PAGE results in largely inactive proteins, some techniques allow *in situ* renaturation followed by specific enzyme detection (for proteases, for example). A potentially powerful development involves a second dimension SDS-PAGE separation of the entire repertoire of protein zones separated in the first dimension after co-stacking with a protease that retains its activity in the presence of SDS. In this way, a comprehensive peptide map of the proteins may be obtained and homologous proteins identified within or between species. Proteolytic fragments, so separated, may also be transferred onto a PVDF membrane and sequenced.

Glycoproteins may be distinguished using the specific periodic acid–Schiffs (PAS) stain whereas phosphoproteins may be detected by intact cell metabolic labelling with $^{32}$P phosphate (see Figure 3) or with [$\gamma$-$^{32}$P] ATP in crude lysates.

## Scanning and Quantification of Individual Protein Zones

Proteins identified by specific or nonspecific staining techniques or by autoradiography or fluorography may be quantified in a relative sense after densitometric scanning of stained gels, photographs or autoradiographs. The inset to **Figure 1** shows the separation of human hair proteins before (lane 4) and after (lanes 1–3) $^{14}$C-*S*-carboxymethylation. Proteins are detected by $^{14}$C autoradiography (lane 1),

Coomassie (lane 2) or silver stain (lanes 2 and 4). The main body of Figure 1 shows a gel scan of lane 2 (red) and lane 4 (blue) as well as the scan of the autoradiograph (lane 1, green) according to the apparent molecular weight of each component in the complex densitometric scan. Readers should refer to the separate article on Instrumentation for Scanning gels for further details in this field.

## Anomalous Migration in SDS-PAGE

The precise structural relationship of SDS to protein during SDS-PAGE is unknown but various studies have indicated that a wide variety of proteins all bind a relatively constant amount of SDS ($\approx$1.4 g SDS $g^{-1}$ protein) and adopt a similar flexible rod shape regardless of their native configuration. It is this supposedly constant very high and uniform charge–mass ratio which allows for reliable molecular weight determination since migration rate therefore depends on molecular sieving alone. Having said this, even the original paper of Laemmli referred to the anomalously slow migration of a bacteriophage point mutant. The very basic proteins, histones, behave so anomalously that special buffer conditions in the presence of urea must be employed to determine reliably their molecular weights. Glycoproteins also show anomalous migration. In this laboratory we have, over the years, seen direct evidence of discrete changes in apparent migration in SDS-PAGE related to single mutations which change the charge on a protein without significantly affecting its calculated molecular weight. **Figure 5**, for instance, shows an SDS-PAGE separation of purified carbonic anhydrase 1 (CA1) from a Caucasian blood donor (lane 1) or an Australian Aboriginal donor (lane 4) who is heterozygous for a polymorphic variant CA1-9. The normal and the variant component of the heterozygote were resolved by isoelectric focusing (lanes 2 and 3, respectively). The variant component differed from the normal component at only one position (Asp $\rightarrow$ Gly) although the apparent molecular weight after SDS-PAGE was 27 kDa compared with 28.5 Da. From this and other previous studies with defined point mutations of defined proteins we propose that each extra negative charge on a modified protein results in retardation on SDS-PAGE such that its apparent molecular weight is greater by 1.5 kDa.We have applied this rule of thumb to changes in the apparent molecular weights of the keratins of human hair after *S*-carboxymethylation which substitutes an extra negative charge for each thiol. See Figure 1 for general molecular weight shift of hair proteins after *S*-carboxymethylation – compare the profile from lane 4 (red) with the profile from lane 2 (blue). Given the published molecular weights of the unmodified keratins we were then able to calculate an apparent thiol content in rough agreement with previous estimations assuming that each thiol group was substituted with an extra negative charge after carboxymethylation (lane 2).

We are therefore convinced that electrophoretic migration of SDS–protein complexes is not totally independent of charge of the native protein and furthermore that if a one-dimensional SDS-PAGE system could be suitably calibrated, the relationship we have described could become useful in studies where the net charge on a given protein is changed genetically or epigenetically in an incremental way.

## Conclusions

Over the past 30 years, one-dimensional SDS-PAGE has become a standard technique in most biological and biomedical laboratories. It offers a powerful combination of resolution (up to 200–300 components in crude mixtures), reliability, versatility (pore size, extraction conditions and buffer systems can be tailored precisely to the conditions required for particular proteins) and reproducibility (different laboratories all refer confidently to certain proteins by their apparent molecular weight on SDS-PAGE, e.g. Hsp 70; see **Figure 4**). In addition, selectivity may be enhanced by using a variety of detection methods ranging from nonspecific staining, metabolic and postmetabolic radiolabelling to highly specific immunological detection after Western blotting. One may confidently expect that one-dimensional SDS-PAGE will continue to play a central role in protein separation in biological laboratories for many years to come, even though Figure 2 suggests that the technology uptake is now stable in that the citation rate peaked in 1990. It may be that improvements in two-dimensional technology (see the article on Two-dimensional SDS-PAGE) and the developing discipline of proteomics involving ever more powerful software to analyse complex two-dimensional protein gels will see a slow erosion of the central position of one-dimensional SDS-PAGE in isolation as method of choice in the resolution of complex protein mixtures.

**See Colour Plate 42.**

## Further Reading

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# Polyacrylamide Gel Electrophoresis

*See* **II / ELECTROPHORESIS / One-dimensional Polyacrylamide Gel Electrophoresis; II / ELECTROPHORESIS / One-dimensional Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis; II / ELECTROPHORESIS / Two-dimentional Polyacrylamide Gel Electrophoresis**

## Porosity Gradient Gels

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### Introduction

The high resolving power of polyacrylamide (PA) gels for proteins, peptides and nucleic acids can be improved by using gradient gels instead of homogeneous (i.e. single concentration) gels. However, a more specific separation of polynucleotides in PA gels affords separation by incorporating a 40–80% denaturant gradient (7 mol $L^{-1}$ urea, 40% (v/v) formamide) into a homogeneous PA gel (of e.g. 6.5% (w/v) total polymer concentration) or applying a temperature gradient to a homogeneous PA gel.

In PA gradient gels the average pore radius decreases with increasing gel concentrations, i.e. in the direction of the migrating protein (polynucleotide) bands. This results in a sharpening of the bands because the molecules at the front of the moving band are slower than those at the rear. Because of this effect, gradient gels need not be covered by a stacking gel, as in disc gel electrophoresis. In porosity gradient gels with a steep increase of polymer concentration (e.g. from 4 to 30% $T$ (w/v) where $\%T$ = g acrylamide + g Bis = $N,N'$-methylenebisacrylamide (Bis) per 100 mL) proteins of a large size range (approximately $10^4$–$10^6$ Da) can be separated. In shallow gradients ($> 4\%$ $T$ to $< 30\%$ $T$), the separable size range of proteins is limited but they still provide an improved band sharpening.

There are two modes to run porosity gradient gels: a fixed-time mode, where electrophoresis is terminated after a certain time, and a time-dependent mode, which means that a number of consecutive electrophoretic mobilities are registered. Fixed-time electrophoresis is performed if protein (polynucleotide) patterns are to be screened, such as in population genetics or when determining the molecular mass of sodium dodecyl sulfate (SDS) denatured proteins. Molecular size properties of nondenatured proteins, however, cannot be elucidated that way, but afford time-dependent investigation of protein mobilities. On the other hand, time-dependent PA gradient gel electrophoresis not only offers the possibility to estimate the molecular mass of native proteins and enzymes but also allows determination of their Stokes radius, frictional coefficient, free electrophoretic